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EDITORIAL

MEAT INSPECTION AND THE ADVANCEMENT OF KNOWLEDGE

In the autumn of 1953 eighty-two people living in the Liverpool area were infected with *Trichina spiralis*. The first warning of the outbreak came as the result of the acute observation of a doctor who on November 6 reported that he had seen a patient whom he suspected was suffering from Trichinosis. This patient was admitted to hospital and the physician's suspicions were confirmed by muscle biopsy. Inquiries were instituted and three more suspicious cases came to light. This was a warning that there might be a more widespread outbreak and the M.O.H. caused a letter to be sent out to medical practitioners describing the clinical features of the cases already seen and requesting to be informed of any further suspected cases. Suspected cases continued to be reported until the third week in November. Patients came from all parts of the city and the following symptoms were shown. Œdema of eyelids, headache, muscular pains, pyrexia, photophobia and insomnia. One death occurred following a myocardial involvement.

In general the clinical picture was that of an exhausting, sometimes painful, disease from which recovery was slow and convalescence protracted. In one instance the patient's dog came under suspicion of being a victim following the gift of a bone. It was violently sick the following day and developed swellings in the region of the eyes and conjunctivitis.

From the medical point of view this outbreak was controlled and managed with great efficiency. Whilst the medical inquiries were being made a serious defect in the meat inspection system was disclosed. It proved to be impossible to trace the pig beyond the abattoir.

Two butchers in widely separated parts of the city each received a half

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carcase of infected meat on October 24. No doubt this diversion of the two parts was responsible for the widespread nature of the outbreak. If the entire carcase had gone to one man it is reasonable to presume that although the number of people infected might have been the same the cases would have been concentrated in a smaller area and control measures easier to put into operation.

To add to the M.O.H.'s difficulty it was found that as no records were kept of individual animal carcases it was impossible to trace their origin and prove or disprove whether the sides of pork came from the same pig.

We hear much about meat inspection. It is a topic of vital importance to every citizen for good health is dependent upon wholesome food. Since the time of that great pioneer Thomas Walley who, almost 100 years ago, asserted that qualified veterinary surgeons were competent to pass judgment on the quality and wholesomeness of meat destined for human consumption the subject has held a leading place in veterinary thought and discussion. Some would assert that the duties of inspection can be done efficiently only by one whose training and examination tests included the study of comparative pathology. The Liverpool incident would tend to give support to this idea but it must also be borne in mind that the recognition of meat infected with Trichinosis is not always an easy task even to an expert pathologist who has to work against time in a busy abattoir. Much of the routine duty of a meat inspector does not call for a profound knowledge of pathology and it can be undertaken by trained technicians whose keen eyes can recognise that which is abnormal. It is essential, however, that the work shall be under the supervision of experts to whose judgment unusual or doubtful cases can be referred. These men being freed from the day to day routine duties could devote their time and skill to enhancing our knowledge of the many disease processes that affect the animal body. A great store of material is at hand and waiting to be garnered, sorted, classified and assessed. The wealth disclosed would be of incalculable value in the fight against disease in humans and in animals. If such a system were put into action accurate records of all the material which passes through the slaughterhouse could be kept. The information made available would be of great scientific value to those who fight disease in the field and in its health and economic repercussions. Disease processes could be traced back to their very beginnings, control measures applied with ease and epidemics such as occurred in Liverpool nipped in the bud quickly after the initial or " marking " case had been recognised. The speedy control of an epidemic of any infections disease depends upon the early and correct diagnosis of the first case and as a rule this is directly proportionate to the wisdom and knowledge of the observer. The prime reason for the inspection of meat is that the people may be supplied with wholesome food. If the work is confined entirely to the slaughterhouse great opportunities for the advancement of our knowledge of disease will be missed. If full advantage be taken of these opportunities the work of the " meat " examiner would be eased and the consumer would benefit in health and finance.

XUM

GENERAL ARTICLES

ISOLATION OF JOHNE'S BACILLI FROM THE UDDERS OF CLINICALLY AFFECTED COWS

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THE only reference in the literature to the isolation of *M. johnei* from milk appears to be that of Alexejeff Goloff (1926). He diagnosed Johne's disease in four cows by finding the bacilli in the fæces and rectal mucosa. One cow died and three were killed. Milk samples from three of the cows were examined during life, precautions being taken against accidental contamination by infected fæces, and Johne's bacilli were demonstrated in the milk of each animal and positive cultures obtained. Johne's bacilli were found on post-mortem examination in the udders and associated lymphatic glands. He claimed also to have isolated the bacilli from the internal organs and blood of all four cows. At the slaughter of one cow during the third month of pregnancy, groups of Johne's bacilli were found in the fœtal envelopes and in the fœtal blood, organs and tissues.

Mathews (1930) isolated Johne's bacilli from the livers of two affected cows; and Dunkin (1938) obtained positive cultures from submaxillary and pharyngeal lymphatic glands. Houthuis (1932) isolated the bacilli from the liver, lungs and portal lymphatic glands of clinically affected cows, but emphasised that they were present only rarely in these sites. We have isolated the bacilli from the spleen and liver of a clinically affected heifer. Dunkin, Glover and Minett (1941) in an investigation of the diagnostic value of 'Johnin' on 104 cattle, many of which were clinically affected with the disease, cultured the intestines and the mesenteric, submaxillary, pharyngeal, bronchial, mediastinal, hepatic and iliac lymphatic glands. Apart from the intestines, and mesenteric lymphatic glands, Johne's bacilli were isolated from an iliac gland only.

Levi (1948) working with artificially infected goats isolated the bacilli from the intestines and associated lymphatic glands, from the tonsils, liver and bile, and from the pharyngeal, submaxillary, bronchial, mediastinal and portal lymphatic glands. He found that artificially infected goats excreted the bacilli in the fæces during the clinical phase of the disease and also during the pre-clinical period.

Taylor (1953) isolated the bacilli from the tissues of a number of naturally infected cows. Cultures were obtained from the liver, spleen and lungs, and from the hepatic, mediastinal, submaxillary, pharyngeal, iliac and prescapular

lymphatic glands. These results suggested that milk from clinically affected cows might possibly be a source of infection and prompted the work here recorded.

Present Investigation

This study was made on 34 clinically affected cows obtained from attested herds. The breeds concerned were Guernseys (20), Jerseys (10) and Shorthorns (4); the preponderance of Channel Island cows is merely a reflection of the principal breeds present in the area from which they came. Twenty-four of the cows were heavily infected, as judged by the number of Johne's bacilli present in the mesenteric lymphatic glands and in the intestinal mucosa; ten were old-standing cases with a few bacilli only in either the mesenteric glands or in the intestinal mucosa. The small number of the bacilli present in many chronic cases is characteristic of the disease.

Technique

Preliminary work on microscopical and cultural examination of milk samples taken during life from clinically affected cows emphasised the danger of accidental fæcal contamination with Johne's bacilli. The udders of affected cows are being continuously splashed with fæces; despite the most rigorous precautions a positive culture obtained from a milk sample, would be open to considerable doubt. On this account, it was decided to kill each animal and to make cultures from the depth of the udder. While this procedure eliminated the risk of adventitious fæcal contamination, it allowed only one examination and did not cover the possibility of intermittent excretion of the bacilli. A detailed post-mortem examination was made of each cow to verify the absence of tuberculosis. The udder was then removed with the supramammary lymphatic glands attached, scrubbed in running water, dried, dipped in methylated spirits and " flamed with gas." Two parallel antero-posterior incisions were made with a sterile knife through the fore- and hind-quarters of each side of the udder for a depth of two to four inches according to the size of the gland and 2 gm. of tissue removed from each quarter. Each sample was ground separately in a Griffith tube, suspended in a 5 per cent solution of oxalic acid and incubated for 30 minutes at 37°C. The suspension was centrifuged and the deposit from each tube sown on phlei-egg media and incubated at 38°C. The tubes were examined for growth at intervals up to six months when, if negative, they were discarded. In addition, a pooled sample of the centrifuge deposits was inoculated subcutaneously into four guinea-pigs to confirm the absence of tubercle bacilli. As a control to the efficacy of the media, a portion of mesenteric lymphatic gland from each animal was treated similarly to the udder tissue and sown on the media; growth was invariably obtained but this varied considerably in amount, presumably according to the number of bacilli present in the inoculum. Smears were made from the supramammary lymphatic glands of each cow and stained by the Ziehl-Neelsen method.

XUM

Positive Cases

Cultures of *M. johnei* were obtained from the udder tissue of two cows.

CASE No. 1.

A seven-year-old Guernsey cow, calved two months. The mesenteric lymphatic glands and the intestinal mucosa were heavily infected with Johne's bacilli.

CASE No. 2.

A three-and-a-half-year-old Guernsey cow, calved some months. Heavily infected similarly to No. 1. Smears of the centrifuge deposit of udder tissue from this cow showed two acid-fast bacilli morphologically similar to Johne's bacilli, and numerous acid-fast granules. Smears of the centrifuge deposits from the other cows were negative. The organisms obtained on culture from these two udders grew very slowly on phlei-egg medium and consisted of acid-alcohol-fast bacilli morphologically indistinguishable from Johne's bacilli; growth was rather meagre on primary isolation but improved on subculturing. Subcultures inoculated on to plain egg and glycerine agar failed to grow. Each strain was inoculated into four guinea-pigs, each guinea-pig receiving a suspension of one-quarter of the growth from a phlei-egg slope; three months after inoculation, they were examined *post-mortem* and all were free from tuberculosis.

Discussion

The thirty-four cows in this series comprised some freshly calved, some in milk, some dry in-calf cows and a few dry, barren cows. Cultures of Johne's bacilli were obtained from the udder tissues of two cows. As positive cultures were not obtained from any of the remaining thirty-two cows, twenty-two of which were heavily infected with the disease and ten were chronic cases, it would appear that the excretion of Johne's bacilli in the milk of clinically affected animals is a relatively rare occurrence; and presumably its presence in the milk of a pre-clinically infected animal would be even rarer. It is probable that in the fulminating type of infection the bacilli may occasionally break through the lymphatic barrier and be carried in the blood stream to various organs. The presence of the bacilli in the udder presumably occurred in this way. Smears of the supramammary lymphatic glands of these thirty-four cows were examined microscopically and in no instance could Johne's bacilli be found; in addition cultures of the supramammary lymphatic glands were made from some of the cows, including the two positive udder cases, but no growth was obtained. Therefore, it appears reasonable to conclude that infection was not carried by the lymph stream. It is conceivable that an occasional calf might contract the disease through milk from an infected udder, but it is improbable that this source is of any practical importance; whereas milk contaminated by the fæces of affected cows is probably a common source of infection. In addition to the cultural examination of udder tissue, milk samples were drawn twice daily for fourteen days from two clinically affected cows, immediately after calving, and sown on phlei-egg medium with negative results.

Conclusions

(1) The udder tissue of thirty-four cows clinically affected with Johne's disease was examined microscopically and culturally for the presence of Johne's bacilli. Positive cultures were obtained from the udders of two cows.

(2) The excretion of Johne's bacilli in the milk of clinically affected cows would appear to be relatively rare and probably occurs only in the fulminating type of infection. It is improbable that this source of infection is of any practical importance in the spread of the disease.

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FURTHER OBSERVATIONS ON NEOPLASMS IN DOGS, WITH PARTICULAR REFERENCE TO SITE OF ORIGIN AND MALIGNANCY

Part 1: Cutaneous, Female Genital and Alimentary Systems

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SOME of the accounts of the incidence of neoplasms in dogs are based on a review of the literature (Courteau, 1935), while others deal with a series of personal observations (Mulligan, 1949*a*). There is a valuable bibliography in the article by Tamaschke (1951/52). There is still a need for the publication of surveys of material examined in different parts of the world, so that we may build up a picture of the occurrence both in space and in time of tumours in dogs, which, besides providing a basis for prognosis, may also throw light on the problem of their ætiology.

A survey has recently been published (Cotchin, 1951) of the 1,211 neoplasms

from dogs that were examined in the Department of Pathology of the Royal Veterinary College, London, during the ten-year period ending December, 1949; during the succeeding four years, a further 1,150 neoplasms from dogs have been examined macroscopically and histologically. In this paper, and in Part II (Cotchin, 1954), it is proposed to deal with the sites of origin of the tumours and with their malignancy as revealed by the presence of metastases. In this series, 261 neoplasms were obtained from dogs on which a post-mortem examination was made by the author and a further 88 were specimens (referred to herein as "post-mortem specimens") taken post-mortem from dogs by veterinary surgeons; it is possible to say with some accuracy, of these 349 tumours, whether or not there were obvious metastases present.

TABLE I

Analysis of 1,150 Neoplasms from Dogs examined during 1950-1953 inclusive

System of Origin	Total number	a	b	Per cent	Per cent in previous series
Skin and subcutis	422	33	5	36.7	42.1
Female genital	266	29	10	23.1	22.2
Alimentary	160	77	25	13.9	16.8
Male genital	77	3	2	6.7	6.9
Lymphatic	75	46	15	6.5	1.1
Skeletal	64	41	9	5.6	3.4
Miscellaneous and uncertain ...	86	32	22	7.5	7.4
	1,150	261	88		

a = the number of cases in which a post-mortem examination was made by the author.
b = the number of cases from which post-mortem specimens were received.

The systems of origin of the 1,150 tumours are shown in Table I, which also shows for comparison the findings in the previous series of 1,211 tumours. It will be noticed that the order of frequency with which the various systems were involved was practically the same in both series, except that neoplasms of the lymphatic system were much more frequent in the present series, and there was also an increase in the proportion of tumours arising from the skeletal system.

A.—Neoplasms of the Skin and Related Structures

A review of some skin tumours in domestic animals, including the dog, is given by Head (1953). The classification of the 422 skin neoplasms in the present series is shown in Table II.

TABLE II

Classification of 422 Tumours of the Skin of Dogs

Epithelial origin:			109
Basal cell and glandular		51	
Hepatoid adenoma (Circumanal adenoma) ...		33	
Non-glandular		25	
Papilloma	2		
Epidermal cyst	6		
Carcinoma	17		
Connective tissue origin:			201
Fibroma	42		
Mast cell tumour	20		
Lipoma	13		
Angioma	10		
Sarcoma and sarcoma-like proliferations	116		
Melanoma:			83
Miscellaneous:			29
			422

1.—Neoplasms of epithelial origin.

(a) *Basal-cell and glandular tumours.*

Of the 51 tumours in this group (which has been studied particularly by Baumgärtner and by Hieronymi, and is reviewed by Richter, 1941), 14 appeared to be of sebaceous gland origin, and 16 of sweat gland origin. The age of the affected animals (known for 42 dogs) ranged from 10 months to 14 years (ave. 8). Nine only of the animals were females. In general, it seems that skin tumours of this group are unlikely to metastasize, although in one case on which post-mortem examination was made, a 5-year-old fox terrier dog showed metastases of an adenocarcinoma, apparently of sebaceous type, from the axillary region to the right inferior cervical lymph node, the lungs, liver and the fascia inside the right thigh.

(b) *Hepatoid adenomas.*

This kind of tumour occurs mainly in ageing male dogs, and has been reviewed by Bruge (1946). Of the 33 hepatoid adenomas examined, all but two occurred in male dogs. Of the 25 animals where it was known, the age ranged from 1 to 14 years (ave. 9); five only of the animals were under 6 years of age. Six of the tumours developed by the side of the preputial opening, one occurred on the back, and three on the ventral side of the root of the tail. None

of these tumours, which otherwise occurred by the anal opening, are known to have metastasized.

(c) *Tumours of non-glandular origin.*

The 25 specimens comprised two papillomas, five and probably six examples of epidermal cysts, and 17 squamous carcinomas. Post-mortem examination was made of an 8-year-old mongrel dog, from which a skin tumour of the leg had been removed some months previously; squamous carcinoma tissue was found in the inguinal node, and a single metastasis was present in the lung. In this series, the squamous carcinomas tended to show marked cornification, and they do not appear to be prone to metastasize readily.

2.—Neoplasms of connective tissue origin.

(a) *Fibromas.*

There were 42 fibromas, occurring in dogs (38 known) ranging from 2 to 15 years of age (ave. 9); 25 of the animals were known to be male, and 16 female. Often in these fibromas there was a whorled appearance suggesting that they were neurofibromas. Post-mortem examination was made on three of the dogs, but no metastases were found.

(b) *Mast cell lesions.*

The neoplastic nature of mast cell lesions is still in doubt. Bloom (1942, 1952), who was the first to describe them, considers that the lesions, which he found to be of frequent occurrence in dogs, are neoplastic and do not disappear spontaneously, and that metastases are not uncommon in the regional lymph nodes, spleen, and liver. The fact that such lesions occasionally recur or spread to local nodes or elsewhere does not of course prove their neoplastic nature. Bloom reported rapid regression of multiple lesions in the skin of an eight-year-old male dog, without recurrence, following cortisone injection. Hangartner (1949), from a study of 11 cases, concluded that the mast cell lesion is a kind of infectious granuloma, as in all the lesions there were chronic inflammatory cells.

The 20 examples in this series affected dogs ranging from 2 to 13 years of age (ave. under 8). There were at least 12 male animals and five female. Of the 17 dogs whose breed was recorded, four were Boxers, three Bull Terriers, and two Bulldogs. In at least four cases, the lesions recurred after operative removal, and in one case, in which the primary lesion occurred on the left hock of an 11-year-old mongrel dog, at post-mortem examination when the dog was destroyed 10 weeks later, local recurrence was noted, and similar lesions were present in the left popliteal, superficial inguinal, deep inguinal and iliac nodes.

(c) *Lipomas.*

There were 13 lipomas, present in dogs ranging from 1½ to 12 years of age (ave. 7½). Seven of the animals were male, six female.

(d) *Angiomas.*

There were 10 specimens, from animals ranging from 1½ to 9 years of age (ave. 6). Five of the animals were male, four female.

(e) *Sarcomas.*

The diagnosis of sarcoma of the skin of the dog is very difficult. McClelland (1947), in discussing the treatment of multiple round cell sarcomas of the skin of dogs, comments on the different opinions expressed by different pathologists of the lesions he was dealing with. Ottosen (1949) found 101 atypical mesenchymal tumours in a total of 201 skin tumours of dogs, and he classified them as being predominantly of three types—reticulosarcoma (67 cases), histiocytic sarcoma (10 cases), and fibrosarcoma (16 cases). Of the reticulosarcomas, only two out of 28 for which information was available were known to be malignant. Ottosen also describes cases of what he calls sarcoma-like R.E.S. proliferations—often multiple skin lesions, for which a granulomatous nature is suggested.

In the present series, 116 tumours were grouped as sarcomas or sarcoma-like lesions. There were 42 fibrosarcomas, occurring in animals ranging from 7 weeks to 15 years of age (ave. 9 years). 20 of the animals were male, 16 female. Post-mortem examination was made in four cases and a further post-mortem specimen was received; none of the cases showed metastases, which agrees with the general experience that fibrosarcomas of the skin of the dog, although capable of recurring, are unlikely to metastasize. Two at least of these tumours appeared to be neurofibrosarcomas.

There were eight examples of the kind called in a previous paper "sarcoids," occurring in young dogs from 5 months to $2\frac{1}{4}$ years of age. Two further tumours were considered to be plasma cell myelomas. The rest of the lesions in this group showed a variable structure, with a tendency to form an "alveolar sarcoma" arrangement of rather large angular cells, often with an admixture of many polymorphs, and the possibility exists that many of these lesions were of myeloid type. Post-mortem examination was made of 11 of the affected animals: six appeared to have lymphosarcomas—all showed involvement, often extensive, of the regional lymph nodes, and in two cases visceral metastases also. The age of the affected animals showing metastases ranged from 6 to 11 years, and it seems that a very cautious prognosis is indicated in sarcomas composed of round cells in ageing dogs.

(f) *Miscellaneous tumours.*

There were 29 tumours in this group, which were not definitely classified. In some, the histological picture suggested a diagnosis of undifferentiated epithelial malignant tumour.

It is proposed to discuss the 83 examples of melanoma of the skin in a separate paper. It may be stated that eight of them were definitely, and 12 were possibly, malignant.

B.—Neoplasms of the Female Genital System

The site of origin of the 266 neoplasms from the female genital system is shown in Table III.

TABLE III

Origin of 266 Tumours of the Female Genital System

Mammary gland	222
Vagina	26
Ovary	14
Uterus	3
Mesovarium	1
	266

1.—Mammary gland.

It is hoped to deal with the mammary tumours, particularly those of the mixed type, in a separate article. Brief mention will be made here of those malignant ones which came from animals on which a post-mortem examination was made, or from which post-mortem specimens were taken.

Eighteen examples of adenocarcinoma were examined. The age of the affected animals ranged from 4 to 13 years (ave. 10). In 13 instances, metastases were present: in the local node (six instances, one being detected histologically only), in the lungs (nine instances, one being detected histologically only), in the bone marrow (two—humerus, femur) and other viscera (four). There was a massive invasion of the underlying abdominal or thoracic wall in six instances.

There were five examples of non-carcinomatous malignant mammary tumours (four post-mortem examinations, one post-mortem specimen). The post-mortem specimen came from a middle-aged bitch, and both carcinomatous and osteosarcomatous tissue were present in the regional lymph node, giving a diagnosis of true malignant mixed tumour. There were two osteochondrosarcomas: the first was found in both posterior mammæ of a 16-year-old mongrel bitch, and had involved the subcutis laterally, the pubic bone dorsally, and showed metastases in the regional nodes and the lungs; the second, involving the fourth and fifth left mammæ of a 11½-year-old mongrel bitch, showed an extraordinary growth of intravascular tumour tissue, in the form of long cords attached to the A-V valve on either side of the heart, with intravascular growth of tumour tissue in the branches of the renal arteries, which had resulted in the production of multiple cortical infarcts. A giant-cell sarcoma was found in the right posterior mamma of a 16-year-old Spaniel bitch, with metastases in the right and left superficial and deep inguinal nodes, right axillary and prescapular nodes, and the spleen. An unspecified malignant tumour was found in most of the mammary glands of a 12-year-old mongrel bitch, with metastases in the left popliteal, right deep inguinal, right and left axillary nodes, lungs, myocardium and kidneys (the brain and bone marrow not being involved).

2.—Vagina.

Amongst the domestic animals, vaginal fibromas and leiomyomas are most

often seen in the bitch. The lesions may be pedunculated or sessile, and, although some of them are well-defined structures, others give the appearance of being hyperplastic rather than neoplastic in nature. The 27 vaginal tumours comprised 14 fibromas, four fibromyomas, three leiomyomas, three lipomas, and three transmissible venereal tumours. The age of the animals affected with fibromas ranged from 5½ months to 10 years (ave. 8).

3.—Ovary.

The 14 ovarian tumours comprised seven granulosa-cell tumours (of which one appeared to be luteinising), three carcinomas, two papillary cystadenomas, and two unspecified malignant tumours. One of the animals with granulosa-cell tumours of the ovaries came to post-mortem examination: a 16-year-old mongrel, which had shown loss of weight for some months despite a fair appetite, had bilateral ovarian tumours—the left ovary measured 15 x 10 cm., the right 6½ x 4½ cm. A 6-year-old Great Dane bitch also showed bilateral granulosa-cell tumours. A 12½-year-old Sealyham bitch showed at post-mortem examination a carcinoma of the right ovary, with secondaries in the renal and hepatic nodes and liver. Post-mortem specimens received were of a carcinoma of the ovary of an aged bitch, a papillary cystadenoma in a 12-year-old Peke and one in a 5-year-old Peke, and an unclassified malignant tumour of the ovary of a 6-year-old Retriever-cross, with secondary tumours in the liver, lung and spleen (possibly an epithelial tumour).

4.—Uterus.

Carcinoma of the uterus is a rare lesion in dogs, and no example was encountered (see Collignon, 1936).

5.—Mesovarium.

A papillary cystadenoma was removed from the mesovarium of a 10-year-old bitch.

C.—Neoplasms of the Alimentary System

The sites of origin of the neoplasms are shown in Table IV. Over half the tumours occurred in the mouth or tonsil region, and a quarter of them in the intestines. The rarity of gastric tumours is once again illustrated.

1.—Mouth tumours.

Apart from 37 tumours of the tonsil, there were 53 others:— gum (34), palate (8), lip (3), buccal mucosa (4), tongue (3), unspecified (1). The types of neoplasm are shown in Table V.

Papilloma of the mouth may be much commoner than might be indicated by Table V, as it is easily diagnosed and histological examination would seem superfluous. Epulides may be considered (Teigeler, 1940) to be either

XUM

TABLE IV

Classification of 160 Tumours of the Alimentary System

Mouth			90
Tonsil	37		
Carcinoma	35		
Lymphosarcoma	1		
Melanoma	1		
Epulis	14		
Melanoma	14		
Sarcoma	11		
Carcinoma	10		
Papilloma	3		
Fibroma	1		
Stomach			2
Carcinoma	1		
Leiomyoma	1		
Intestines			43
Lymphosarcoma	33		
Carcinoma	6		
Leiomyoma	1		
Leiomyosarcoma	1		
Papilloma	1		
Spindle cell sarcoma	1		
Liver			17
Liver cell tumour	9		
Bile duct tumour	7		
Lymphosarcoma	1		
Pancreas			8
Carcinoma	8		
			160

TABLE V

		Gum	Palate	Lip	Buccal mucosa	Tongue	Unspecified
Epulis	14 ...	14	—	—	—	—	—
Melanoma	14 ...	5	3	2	4	—	—
Sarcoma	11 ...	6	3	1	—	1	—
Carcinoma	10 ...	7	1	—	—	2	—
Papilloma	3 ...	2	—	—	—	—	1
Fibroma	1 ...	—	1	—	—	—	—

simple neoplastic growths on the gum, which may be benign or malignant, or inflammatory epulides, often associated with irritation by a tooth which may be misplaced, or they may be disorders of development. It is probable that the 14 epulides listed above may have been mostly granulomatous rather than neoplastic in nature, but it is useful to include them for comparison with the incidence of malignant tumours: it would seem that, when in doubt, a section of a gum lesion is indicated.

Post-mortem examination was made on 12 of the dogs with mouth tumours, and post-mortem specimens were examined from five others. Seven of the dogs on which post-mortem examination was made had malignant melanomas (buccal mucosa 3, palate 2, lip 1, gum 1) and a further post-mortem specimen was of a malignant melanoma of the gum. Other dogs autopsied had (i) carcinoma of the hard palate, with penetration of the hard palate and spread to the orbit, (ii) sarcoma of the hard palate, with three lung metastases (this dog also had lymphatic leukosis), (iii) carcinoma of the gum, (iv) sarcoma of the gum, and (v) sarcoma of the tongue, respectively. The melanotic neoplasms of the mouth will be described in a separate paper, but there were indications that they were all malignant. Three of the post-mortem specimens were epulides, the fourth was a melanoma, and the fifth was a carcinoma of the tongue of a 7-year-old male Pomeranian. This animal had a history of at least 7 weeks' duration of inability to chew food, and a bad odour from the mouth, with progressive emaciation. The tumour appeared macroscopically as an irregular gap with thickened borders on the right antero-lateral border of the tongue. Another tongue tumour, in a 4-year-old bitch, was thought to be an anaplastic carcinoma.

Of the 37 tonsil tumours, 25 were obtained at post-mortem examination, while a further three were post-mortem specimens. All but two of the tumours (a lymphosarcoma in a 5-year-old cross-bred terrier dog, and a melanoma in a 12-year-old Sealyham dog) were carcinomas of the surface epithelium, and once again it is found that this tonsil tumour is the most important single specific carcinoma that is seen here in dogs. In 11 cases, the left tonsil was affected, in 22 the right; 24 of the animals were known to be males, 13 females. The age of the affected animals ranged from 2 to 13 years (ave. $8\frac{1}{2}$). Metastases were present in no less than 26 of the dogs: the metastases were mainly distributed in the lymph nodes (mandibular: ipsilateral 10, contralateral one; superior cervical: ipsilateral 20, contralateral eight; prescapular: ipsilateral nine, contralateral one; bronchial node: three; anterior mediastinal node: one; suprasternal node: one), but involved the lungs in six cases, and the liver in one. In one case, both thyroid glands contained metastases; in another, one thyroid gland was involved

The clinical symptoms—which included difficulty in eating and swallowing, salivation, and loss of condition—had been present for periods which varied from one to 16 weeks in duration, averaging six weeks. The prognosis for carcinoma of the tonsil appears to be poor, owing to the early and common nodal involvement.

XUM

2.—Œsophagus.

No œsophageal tumour was seen. While carcinoma of the œsophagus has been recorded in the dog, it is a very rare lesion. Krogh (1952) gives an interesting account of a case of metastasizing carcinoma of multicentric origin in the œsophagus associated with diffuse œsophagitis in a 7-year-old male dog, which had shown bloody vomiting for 2 to 3 months, and an inability to take solid food. The œsophagus showed in its upper and lower thirds diffuse neoplastic infiltration together with smaller well-defined tumours. The neoplasm had spread to the stomach wall and trachea, and the middle cervical nodes.

3.—Stomach.

Carcinoma of the stomach is a rare lesion in dogs. Campbell and Lauder (1952), in six years at the Glasgow Veterinary School, saw 66 carcinomas in dogs, of which two affected the stomach. In this series, two animals only showed neoplasms of the stomach. A 12-year-old mongrel, destroyed with a fibrosarcoma of the subcutis of the right thigh, was found incidentally to show five leiomyomas of the stomach wall. The second case was of a 4½-year-old Scottie bitch, which had vomited two or three times daily for some six months. There was a progressive loss of condition, and the animal was destroyed after exploratory laparotomy. On the lesser curvature of the stomach, there was an ulcer of the mucosa, some 5 cm. long and 2½ cm. wide, with well-defined edge and a fibrous floor. Histologically, carcinomatous tissue was seen in the submucosa and muscularis, but a definite origin from and continuity with the gastric mucosal glands was not observed.

4.—Intestines.

Ten of the 43 specimens comprised five and probably six examples of carcinoma (small intestine one, colon one, rectum three and probably four), a leiomyoma of the anal region in a 14-year-old male dog, a leiomyosarcoma of the small intestine of a 4-year-old fox terrier bitch, a papilloma of the small intestine removed at operation from a 3-year-old dog, and a spindle cell sarcoma of the jejunum of a 9-year-old terrier-cross dog.

Two of the dogs with carcinoma of the rectum, and one with carcinoma of the colon, were examined post-mortem. An 8-year-old mongrel bitch had been showing loss of condition, with tenesmus, passage of blood-stained fæces and only fair appetite, for eight weeks before destruction. An adenocarcinoma was present in the colon about 4 inches from the anal rim, with multiple metastatic deposits under the serosa of the ventral part of the peritoneal cavity, on both surfaces of the diaphragm, on the surface of the stomach, in the broad ligaments of the uterus, in the great omentum, on the mesentery, in the mediastinum, and on the pericardium. A second tumour was in a 9-year-old mongrel bitch, which had been showing similar symptoms before it was destroyed. Three irregular papillomatous deposits of adenocarcinoma were found just inside the anal rim, with metastasis in the colonic lymph node, and,

in accord with the invasion of hæmorrhoidal veins seen in section, an extraordinary diffuse infiltration of the lung. Finally, a 6-year-old Airedale bitch had been showing tenesmus and passage of blood-stained fæces for two months prior to destruction; a diffuse annular thickening of the rectal wall, about 2 cm. long, was found just behind the level of the entrance to the pelvis, and showed histologically marked infiltration of the rectal wall by a mucoid adenocarcinoma, with metastasis in the rectal lymph node.

Three of these ten tumours were post-mortem specimens—a rectal carcinoma, a leiomyosarcoma and a spindle-cell sarcoma of the small intestine.

Thirty-three of the 43 intestinal neoplasms were lymphosarcomas. The affected dogs ranged from 2 to 12 years of age (ave. 6½). 19 were known to be male, 11 female. 24 of the dogs were examined post-mortem, and post-mortem specimens were received from three others. The symptoms noted included inappetance (14 dogs), marked (12) or slight (1) loss of condition, vomiting (8), diarrhœa with (5) or without (3) blood, a palpable abdominal mass (4), thirst (4), listlessness (4), tenesmus (in 4 dogs with rectal neoplasms), fever (2), abdominal pain (1) and anæmia (1). The lesions occurred most often in the small intestine, but the large intestine was involved in 10 cases (including seven lesions of the rectum), and both small and large intestine were involved in a further three animals. In the small intestine, the chief site of lesions was the jejunum, but in seven cases the duodenum was involved, and in three cases the ileum. The lesions in at least 11 cases were multiple, ranging in number from 2 to 7 in the small intestine to many in the large. The lesions were very diffuse or annular thickenings of the gut wall by a grey tissue, which usually faded rather sharply at the edges into normal tissue. Due to involvement of the muscularis, the lesions tended to be more or less diverticulate, with local adhesions of the omentum. The origin of the lesions from Peyer's patches or from solitary follicles was sometimes strongly suggested.

The chief site of secondary tumour formation in these cases of intestinal lymphosarcoma was the mesenteric lymph node, which was involved in at least 16 cases, and measured up to 9 x 7 cm. on the cut surface. The liver was involved in five cases by discrete and diffuse deposits. Other nodes involved were the gastric (5 dogs), suprasternal (6), hepatic (4), anterior mediastinal (3), ileocolic (3), bronchial, posterior mediastinal, rectal, splenic, pancreatic, and pericardial (each 2). The renal pelvis, spleen, myocardium, lung and thymus were each involved in single cases. Unfortunately, no information is available as to the blood or bone marrow picture in these cases.

5.—Liver.

(For an account of liver tumours in mammals, see Messow, 1952/53).

Of the 17 specimens five were obtained at post-mortem examination and nine were post-mortem specimens. The affected animals ranged from 5 to 14 years of age (ave. 9½). There were at least 13 females and three males. Nine of the tumours were derived from liver cells, six being thought to be adenomas and three carcinomas from their histological appearance. Three, and probably

four, were derived from bile-duct epithelium, and there were three others which were adenocarcinomas; these latter may have been of bile-duct origin, but they were in bitches, and may possibly have originated from the mammary gland. One tumour was a lymphosarcoma.

The chief symptoms were the loss of condition, varying from slight to marked, and swelling of the abdomen with a palpable abdominal mass. Three cases showed diarrhœa, two thirst, two vomiting, one ascites, and only one slight jaundice. A 5-year-old bitch died suddenly following hæmorrhage from a ruptured hepatic adenoma—such tumours tend to be very large growths, soft and friable, occupying the ventral part of one lobe. The carcinomas were multiple, and this tendency to primary multiplicity makes it sometimes difficult to decide whether an adenocarcinoma in the liver is primary or secondary. Mulligan (1949*b*) in reporting four cases of primary liver-cell carcinoma from dogs, found only eight cases of explicit bile-duct tumours in 35 cases of liver tumours recorded in the literature.

6.—Pancreas.

Nodular hyperplasia of the pancreas is quite common in ageing dogs but the lesion appears to be of no clinical significance. Carcinoma of the pancreas is mostly an exocrine lesion in dogs, although Hansen (1949) has described neoplasia of the endocrine tissue. In this series, six pancreatic tumours were obtained post-mortem, and the two others were post-mortem specimens. They all appeared to be adenocarcinomas, although there is some doubt in three cases that the lesions were primarily of pancreatic origin. All the six cases examined post-mortem showed metastasis—in the liver (5 dogs), lungs (4), omentum (3), mesenteric node (2), diaphragmattic serosa (2), suprasternal node, spleen, myocardium, mesocolon, and both ovaries (once each). The tumours occurred in dogs ranging from 4 to 11 years of age (ave. 8). The symptoms recorded were loss of appetite (4 dogs), loss of condition, abdominal swelling, slight jaundice (2 each), thirst, rapid breathing, vomiting, salivation, and anæmia (one each). The complex clinical history in a 5-year-old Boxer dog from which a post-mortem specimen was received was apparently due to obstruction by the tumour of the pylorus, bile-duct and pancreatic duct.

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XUM

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MALIGNANT NEOPLASIA AFTER ANTI-RABIES INOCULATION

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THE sequelæ of malignant neoplasia after inoculation with anti-rabies vaccine in two dogs is here recorded. The vaccine used was a 20 per cent brain-emulsion in glycero-saline with 5 per cent phenol as preservative. Both animals had been vaccinated every year with the same type of anti-rabies vaccine. In the case of the Alsatian the injections had been made at the same site as in previous years.

Case 120/XXIX. "Diana," 8-year-old, female, silver-grey Alsatian. Given anti-rabies inoculation every year, last injection being done 10 months ago, over the dorsal angle of the scapula. The growth first appeared in August, 1951. The dog was presented for examination and treatment in September, 1951, as the growth was increasing in size rapidly.

The dog was in good condition and its appetite was excellent. It was bright and alert, very playful, and all functions were normal. The heart and pulse were good and visible mucous membranes were normal. Skin condition was good but over the growth an area had been scratched raw and there was an ulcerated surface.

Under 2 per cent Novotux local anæsthesia, using 8 c.c. to infiltrate the superficial and deep areas, a tumour (4 cm. x 3 cm. x 1½ cm.) was enucleated.

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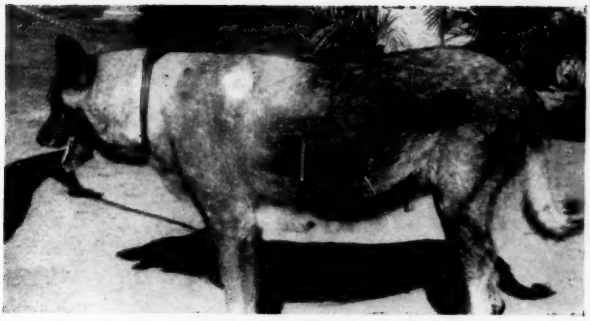

Fig. 1. The animal one month after operation.

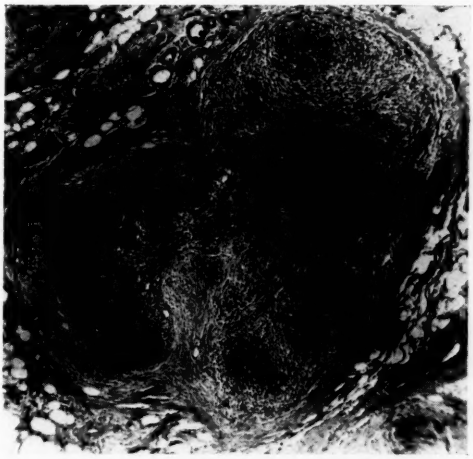

Fig. 2. Low power figure x38 showing many whorled structures.

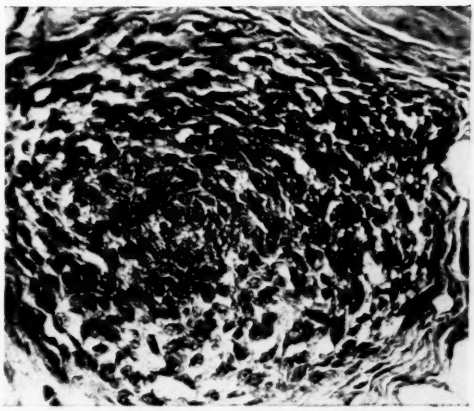

Fig. 3. High power figure x336 of a "whorl."

(*Article by Brito-Babapulle, page* 230)

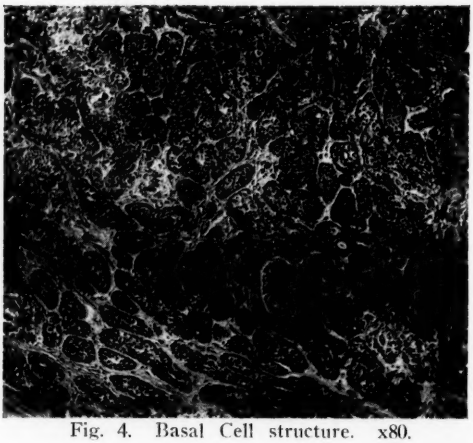

Fig. 4. Basal Cell structure. x80.

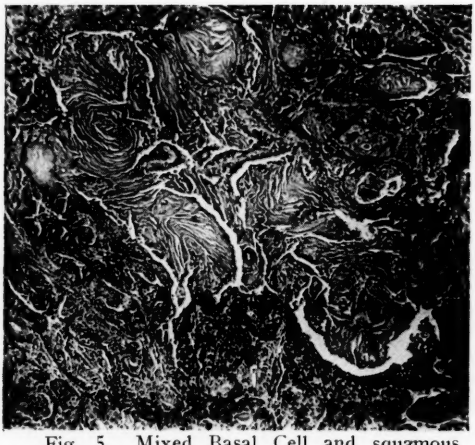

Fig. 5. Mixed Basal Cell and squamous carcinoma. x80.

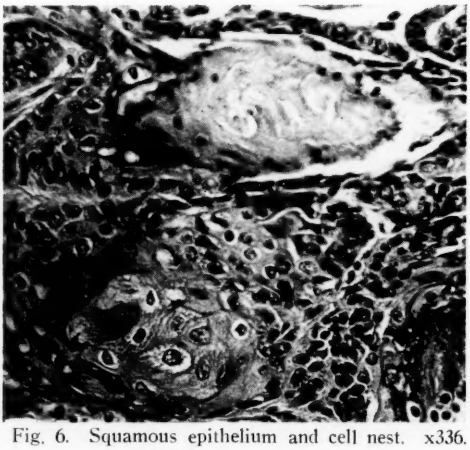

Fig. 6. Squamous epithelium and cell nest. x336.

(All microphotographs by Mr. G. Webster, Department of Forensic Medicine, University of Ceylon).

(*Article by Brito-Babapulle, page* 230)

XUM

An external packing of silver proteinate (Protargol) and two sutures were applied. The healing was rapid. The sutures were removed one week later and the photograph (Fig. I) shows the dog one month after operation. Two years after the operation the dog was seen by me but no recrudescence of tumefaction was observed. The dog had been inoculated with avianised rabies virus (Lederle) six months prior to the the date of my seeing it last.

The tumour was examined by Professor W. A. E. Karunaratne, Professor of Pathology, Faculty of Medicine, University of Ceylon, who reports as follows:

" Fifty-two M.C. The section is that of a tumour showing a characteristic ' whorled ' arrangement of cells as seen in the endotheliomatous varieties of human meningiomas. The tumour is divided into lobules of varying sizes by strands of connective tissue, some containing one or two whorled structures, whilst others show several such structures. The whorls are quite solid, but no calcified sphærules were found. Mitotic figures are frequently seen, and there is a fair number of thin-walled vessels. Mucoid degeneration is evident in a few areas. There is a moderate amount of fibro-fatty tissue between the lobules. The section is that of a malignant tumour showing the histological structure of an endothelioma with a certain degree of mucoid degeneration."

Case 331/XXIX. " Dulcie," a brown Dachshund aged two years. Anti-distemper vaccine was given at six months of age. Anti-rabies vaccine, first injection in March, 1951. Second injection in June, 1952. Growth appeared in July, 1951, as a small pea-sized subcutaneous nodule that was sessile and stationary in size. After the second injection of anti-rabies vaccine the growth started to increase in size.

The dog was presented for examination in July, 1952. It was in good condition and all bodily functions were normal. The skin was in good order and appetite was normal. The growth was located in the paralumbar area on the right side. It was the size of a walnut, hard and not painful on palpation or squeezing.

Local anæsthesia 2 per cent Novotux 5 c.c. was used for infiltration and enucleation of the growth. A dressing of Proflavine hemisulphate and penicillin powder was applied. One suture was inserted and recovery was uneventful. The growth was sent to the same pathologist as in the previous case and the following report was made:

" Forty M.C. This section is that of a baso-squamous carcinoma. Most of the tumour tissue is of the basal cell type, but a few squamous elements including cell nests are also present."

Subsequent history. Six months later the bitch was presented by the owner because of a sanious vulval discharge and a suspected prolapsus vaginæ. Per vaginam examination revealed multiple growths of Infective Venereal Granuloma. Under anæsthesia all of the growths in the urogenital sinus were extirpated. The mucous membrane curetted and a protargol bougie inserted daily for 10 days.

The owner reports that the dog is getting on well (February 2, 1954).

Discussion

Anti-rabies vaccination has been done in Ceylon for well over 20 years. To the best of my knowledge there are no records of malignant neoplasia occurring after its administration, these two being the first records of such growths. There appear to be no common histological features between the two tumours, one being a baso-squamous carcinoma and the other an endothelioma or neurofibroma, and the carcinogenetic effect of the vaccine, or initiator value of the vaccine as a carcinogen is therefore doubted. The only possible effect that it could have produced appears to be, a co-carcinogenic or promoter effect, on cells which have previously undergone change on account of the action of an unknown "initiator."

Various substances have been associated with "promoter" action such as tar products, dibenzpyrene, methyl-cholanthrene, calcium compounds, tobacco in the clay pipe, the betel nut, nitrogen-mustard, mustard gas, X-rays, deep-rays, etc. In the anti-rabies vaccine used there are lipidal substances from the brain substance, an attenuated rabies virus—which, like all viruses, is sometimes crystallisable, molecules of nucleoproteins—and phenol as a preservative. Chromosome production and protein synthesis are dependent on nucleic acid and when nucleic acid is excessive the rate of mitosis is uncontrolled and malignancy results in the susceptible (Darlington, 1945). This is thought to be due to an interference with desoxyribonucleic acid, the basic cell component necessary for normal cell division, that is found in all cells (Science News Letter, 1951).

Acknowledgment

The appreciation of valued suggestions by Professor G. H. Cooray is acknowledged.

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STUDIES ON ETHIDIUM BROMIDE

IV. The Toxicity of Ethidium Bromide for English Cattle*

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DIMIDIUM bromide (2:7 diamino-10 methyl-9 phenyl phenanthridinium bromide) has been used for the treatment of trypanosomiasis in African cattle for some time. Thorold and Plowright (1952), Plowright, Burdin and Thorold (1952), Plowright and Burdin (1952), and Burdin and Plowright (1952*a*), have shown that in a dosage of 3 mg/kg this drug will produce a delayed toxicity syndrome affecting the liver of cattle, characterised by anorexia and loss in weight, increase in plasma alkaline phosphatase activity and a positive van den Bergh reaction.

Burdin and Plowright (1952*b*) and Burdin (1953) have studied other phenanthridinium compounds including ethidium bromide (2:7 diamino-10 ethyl-9 phenyl phenanthridinium bromide) and suggest that for this delayed toxic syndrome to develop it is necessary that there should be a methyl group in position 10 (the quaternary nitrogen atom) and amino groups at positions 2 and 7. They suggest that the toxic syndrome may be related to interference with biological methylation.

Carmichael (1950) studied the toxicity of dimidium bromide for English cattle and found no signs of liver damage as measured by the van den Bergh test, he suggested that for toxicity to develop there must be some interaction between the drug and an undefined factor peculiar to parts of Africa.

The work to be described was undertaken with a view to studying the toxic effects, if any, of ethidium bromide but two animals were given dimidium bromide for comparative purposes. Our results suggest that the effect is largely due to the drug, as a syndrome similar to that described in East Africa was observed in dairy cattle kept under English conditions. These results also confirm the finding of Burdin (1953) that ethidium bromide is considerably less toxic than dimidium bromide.

Materials and Methods

Twelve cows were used in this experiment selected from the dairy herd attached to the laboratory. At the commencement of the observations they were in varying stages of lactation. As little was known about the normal levels of alkaline phosphatase and bilirubin, regular weekly observations of these were made for a period of about two months before dosing. Two animals were kept as controls and the remainder were dosed subcutaneously with either dimidium bromide or ethidium bromide in accordance with the scheme shown in Table I. Following the administration of the drug the weekly observations were continued, extra observations being made as required, for a further three months. As a

* Parts I and II of this series were published in *The Veterinary Record*, Vol. 65, 589 and 907. Part III was published in *The British Veterinary Journal*, Vol. 110 (3), 96.

TABLE I

Cow's Name	Wt. kg.	Drug	Dose mg/kg. b.w.	Total dose in grams
Ada	557.8	Ethidium bromide	10	5.6
Bunty	557.8	Ethidium bromide	10	5.6
Connie	444.4	Ethidium bromide	5	2.2
Olive	439.9	Ethidium bromide	2.5	1.10
Wilma	621.3	Ethidium bromide	2.5	1.55
Yvonne	507.9	Ethidium bromide	2.5	1.3
Fairy	553.3	Ethidium bromide	1	0.55
Ida	507.9	Ethidium bromide	1	0.51
Veda	530.6	Dimidium bromide	3.0	1.60
Zena	603.2	Dimidium bromide	3.0	1.81
Clara	557.8	Control	—	—
Winnie	480.7	Control	—	—

further test of liver function the Bromsulphalein Clearance was determined on certain of the animals. Blood samples were collected from the jugular vein into bottles containing heparin and the plasma alkaline phosphatase activity and bilirubin determined by the method of King (1947). Bromsulphalein Clearance was determined by the technique described by Goodman (1952). Changes in milk production were obtained from the normal daily milk records.

Results

Following the injections there was a considerable local reaction in all the animals; this took the form of swellings, the degree of swelling being proportional to the dose administered. In only one case, that of an animal receiving 10 mg/kg of ethidium bromide did the swelling proceed to the stage of necrosis. In all the other cows the swellings gradually subsided. The cow which showed the greatest degree of swelling also showed loss of appetite for a few days but this was probably due to the physical difficulty in reaching her food.

None of the animals which were in milk showed any marked loss of milk following the injection with the exception of the cow showing the greatest degree of swelling and this cow dried off rather more rapidly than would have been expected.

Only three other animals showed any further clinical symptoms. These were the two animals receiving 3 mg/kg dimidium bromide and one of the animals which received 10 mg/kg ethidium bromide (Ada).

The first animal to show clinical symptoms was Zena (3 mg/kg dimidium). Anorexia was noted on the 35th day after treatment and the cow commenced to lose condition rapidly. She continued to lose condition, ate very little and was destroyed in extremis on the 56th day after dosing. Veda (3 mg/kg dimidium) and Ada (10 mg/kg ethidium) went off their food 41 days after dosing and lost condition for about a week. After this they made a fairly rapid recovery. Three to seven days after the appearance of clinical symptoms these three animals gave a positive direct van den Bergh reaction. This reaction remained positive

in Zena (3 mg/kg dimidium) until she was killed, the intensity of the reaction increasing all the time. In the other two animals Veda (3 mg/kg dimidium) and Ada (10 mg/kg ethidium) the van den Bergh reaction became negative again after four days. None of the cows gave positive van den Bergh reaction, direct or indirect during the preliminary observations.

The three clinically affected animals also showed a sharp rise in plasma alkaline phosphatase activity very shortly after the appearance of symptoms. The rise reached its maximum between the 44th and 55th day. The second animal, Bunty, which received 10 mg/kg ethidium, exhibited the greatest degree of local reaction and did not show any delayed clinical symptoms nor did she give a positive van den Bergh reaction; however, about the 42nd day there was a rise in plasma alkaline phosphatase activity which reached a maximum on the 48th day and then subsided.

From the beginning of the preliminary observations one cow, Wilma, showed a steady rise in plasma alkaline phosphatase activity which reached a maximum just before calving, after which it fell fairly rapidly.

The Bromsulphalein Clearance measures the volume of plasma cleared of the dye in a given time. It has been shown that for practical purposes Bromsulphalein is removed by the liver, so a low Bromsulphalein Clearance is indicative of impaired liver function.

The Bromsulphalein Clearance was determined on the two control animals and on Ada (10 mg/kg ethidium) and Veda (3 mg/kg dimidium) on the 55th day. Both Ada and Veda had shown clinical symptoms, positive van den Bergh reactions and increased plasma alkaline phosphatase levels, but when the Clearances were determined, the van den Bergh reactions were negative and the plasma alkaline phosphatases had passed their maximum levels. Clearance was much more rapid in the two control animals than the two affected animals.

The results obtained are summarised in Table II.

TABLE II

Cow	Drug and dose	Plasma Alkaline Phosphatase Activity Units per ml Pre-dosing Maximum	Post dosing Maximum	Days to max. level	Days van den Bergh Positive	1st appearance of clinical symptoms	Bromsulphalein clearance at day 58 in ml/min
Ada	10 mg/kg Ethidium ...	7.5	17.6	44	44, 48	41	6.8
Bunty	„ „ „ ...	7.7	15.8	48	neg.	—	
Connie	5 „ „ ...	9.3	10.7	70	„	—	—
Olive	2.5 „ „ ...	5.6	7.3	48	„	—	—
Wilma	2.5 „ „ ...	28.0*	18.5	41	„	—	—
Yvonne	2.5 „ „ ...	11.6	11.2	55	„	—	—
Fairy	1.0 „ „ ...	8.0	6.3	41	„	—	—
Ida	1.0 „ „ ...	5.5	5.0	42	„	—	—
Veda	3.0 mg/kg Dimidium ...	13.6	17.7†	55	48, 51	41	8.54
Zena	3.0 „ „ ...	7.6	2.7	48	38 to death	34	—
Clara	Control ...						30.6
Winnie	Control ...						20.0

*This value was obtained about 4 days before calving, there was then a fairly rapid fall.
†On 35th day level was 5.1.

Zena (3 mg/kg dimidium) was killed in extremis on the 56th day after dosing. On post-mortem examination it was seen that the mucous membranes were icteric while the liver was pale, yellow in patches and fairly hard to the touch. The cut surface was more yellow and very greasy. The gall bladder was grossly engorged with bile although the bile duct appeared to be patent. There were adhesions to the body wall at the site of a previously attempted biopsy. The rumen contained little food and showed no sign of fermentative activity. The kidneys were finely mottled and slightly congested. Apart from some yellow staining of the valves, the heart was normal.

Portions of the liver and kidney were fixed in acetone or normal saline. It is of interest to note that the acetone extracted large quantities of pigment from the portions of liver. Examination of appropriately stained sections showed fatty changes in the periphery of the lobules of the liver with increased reticulin and fibrosis. Sections of the kidney showed fatty changes in the cells lining the tubules.

Discussion

The pattern of plasma alkaline phosphatase activity and van den Bergh reactions, the post-mortem appearances and the histopathology observed in Zena (3 mg/kg dimidium) leave little doubt that this animal developed the delayed toxicity syndrome first described by Thorold and Plowright (1952) and subsequently studied by Plowright, Burdin and Thorold (1952), Burdin and Plowright (1952*a* and *b*) and Burdin (1953).

Veda (3 mg/kg dimidium) and Ada (10 mg/kg ethidium) showed a similar pattern of plasma alkaline phosphatase activity and van den Bergh reactions and a much reduced Bromsulphalein Clearance, suggesting that they too had suffered from a similar delayed toxicity affecting the liver. The other cow which received 10 mg/kg of ethidium bromide showed a raised alkaline phosphatase activity at about the same time as the other animals but showed no clinical symptoms and no positive van den Bergh reaction.

From these results it would appear that dimidium bromide in a dose of 3 mg/kg is capable of giving rise to the same delayed toxicity syndrome in English dairy cattle as that described in East Africa by Burdin and his coworkers and Ford *et al* (1953) in the Sudan. The results with ethidium bromide are in accord with the results of Burdin (1953) who has found in East Africa that this drug is less toxic than dimidium bromide.

Carmichael (1950) studied the effect of dimidium bromide on English cattle (4 mg/kg) and found no evidence of liver damage as revealed by negative indirect van den Bergh reactions. He suggested that nutritional factors may play a part in the development of toxic symptoms following the administration of the drug. As in the present experiment one cow, Bunty, (10 mg/kg ethidium) showed an increased plasma alkaline phosphatase activity without a positive van den Bergh reaction, it may be that the van den Bergh reaction alone is not sufficiently sensitive to detect the liver damage.

Burdin and Plowright (1952) have suggested that the toxicity of dimidium

bromide may be due to interference with the biological methylation process, as the drug has a quaternary nitrogen atom in it. The results of this experiment are in accord with this view, though it might have been expected that owing to the better diet of English cattle there would not be the same likelihood of deficiency of vitamins concerned in these metabolic reactions.

A further point of interest is that in the cows which showed signs of liver damage by a positive van den Bergh reaction, the clinical symptoms of anorexia and loss of condition were observed before biochemical changes could be demonstrated.

This finding is perhaps not so extraordinary when the very large mass of active tissue constituting the liver is considered, and also its manifold functions. There may well be sufficient active tissue left to deal with pigment metabolism when there is insufficient for more important functions. This suggestion is supported by the fact that despite the van den Bergh reaction and the plasma alkaline phosphatase activity having returned to normal levels, two affected cows still showed a markedly diminished Bromsulphalein Clearance.

Acknowledgments

I should like to express my thanks to Mr. D. L. Hughes, F.R.C.V.S., Dip.Bact. (Lond.), Head of the Veterinary Science Division for his interest in this work; Miss B. Eastwood, B.Sc.; and Miss M. Norman for technical assistance; Mr. L. Kelk for looking after the animals; and to the directors of Boots Pure Drug Co., Ltd. for permission to publish.

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STUDIES ON DISINFECTION OF EGGS AND INCUBATORS

III. The Use of Formaldehyde at Room Temperature for the Fumigation of Eggs Prior to Incubation

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Introduction

WILSON (1951) has shown that 1.5 c.c. of formalin added to 1 gram of potassium permanganate per cubic foot of incubator space ($5\frac{1}{4}$ oz. to $3\frac{1}{2}$ oz. per 100 cu. ft.), when used under conditions of room temperature and humidity, was capable of killing *S. typhi-murium* on the surface of the egg shell after 30 minutes' exposure.

In the previous paper in this series (Lancaster and Crabb, 1953) results were given of the formaldehyde gas concentration which was required to kill *S. pullorum* on the surface of fresh whole eggs under normal incubator conditions (100° F. dry bulb and 85° F. wet bulb). When using the method of gas analysis described, a minimum concentration of approximately 6.5 milligrams of formaldehyde per cubic foot was required to kill *S. pullorum* on the surface of the hen's egg. The quantity of formalin necessary to produce this concentration was found to depend partially on the degree of airtightness of the incubator. For example, in a completely sealed incubator $1\frac{1}{2}$ oz. formalin per 100 cu. ft. (0.42 c.c. per cu. ft.) gave a gas concentration of 10 mg. per cu. ft.

An investigation has now been completed on the efficiency of formaldehyde at room temperature and room humidity. Many hatcheries already use pre-incubation fumigation chambers and the problem has arisen as to whether it is necessary to bring the eggs in the chamber to 100° F. before fumigation or whether formaldehyde can be used satisfactorily at room temperature. It was also considered of interest to determine the concentration of formaldehyde necessary to kill *S. pullorum* under these conditions using the method of gas analysis described in the previous paper of this series.

Methods

Incubator fluff and debris were not used in the present work, since they play no part in the problem of pre-incubation fumigation.

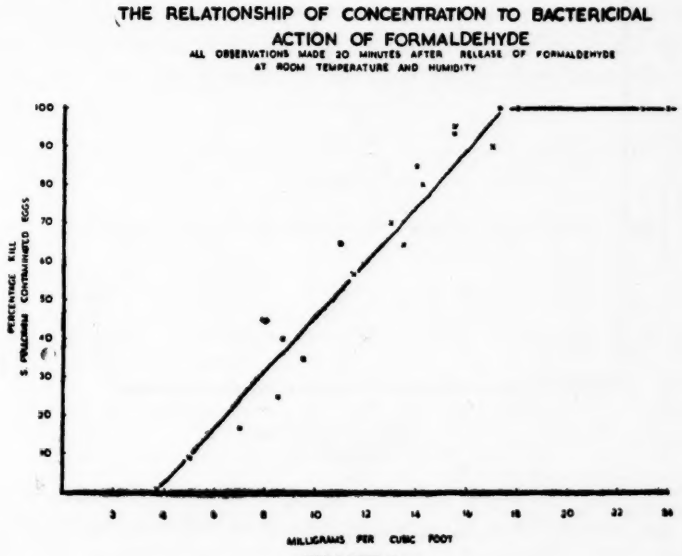
Fresh clean uncracked eggs were contaminated and subsequently examined according to the methods previously described (Lancaster and Crabb, 1953). One of the sealed incubators used in the previous work was modified for use in the present investigation, by switching off the heating elements but leaving the fan connected. In this way, circulation of the formaldehyde was ensured, whilst the incubator remained at room temperature.

Gas samples were taken at intervals and the formaldehyde concentration estimated according to the method already described (Lancaster and Crabb, 1953).

XUM

Results

(a) *The concentration of formaldehyde required to kill S. pullorum after 20 minutes' exposure. (Graph I).*



GRAPH I

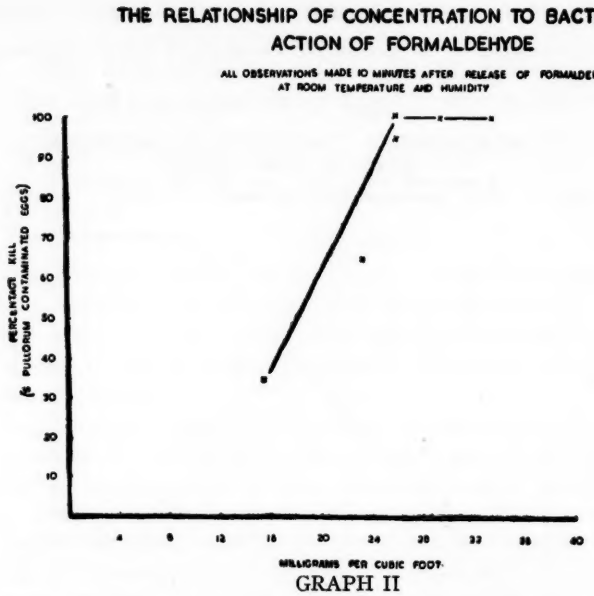
These experiments were carried out in a manner similar to that described in the previous publication in this series. The results, which are shown in Graph I, indicate that a minimum concentration of 17 mg. of formaldehyde per cu. ft., using $3\frac{1}{2}$ oz. formalin per 100 cu. ft. (1 c.c. per cu. ft.), was necessary for the complete destruction of *S. pullorum* on contaminated eggs at room temperature. The results also indicate that a decrease in formaldehyde concentration produced a proportional decrease in the percentage of eggs rendered free from *S. pullorum*.

(b) *The concentration of formaldehyde required to kill S. pullorum after 10 minutes' exposure. (Graph II).*

Under normal hatchery management it is often necessary to place large numbers of fresh eggs into the incubators within a relatively short period of time. Under these conditions, the size of the fumigation chamber is often insufficient to deal quickly with numbers of eggs to be fumigated prior to transfer to the incubators.

It was therefore considered important to find the concentration of formaldehyde which was capable of killing all *S. pullorum* on the surface of the hen's egg within an exposure time of 10 minutes.

The results, which are shown in Graph II, indicate that to be effective a higher gas concentration is required at a 10-minute exposure than at a 20-



GRAPH II

minute exposure. To kill *S. pullorum* during a 10-minute exposure it required a final gas concentration of approximately 25 milligrams formaldehyde per cubic foot. The addition of $5\frac{1}{4}$ oz. formalin to $3\frac{1}{2}$ oz. potassium permanganate per 100 cu. ft. (1.5 c.c. to 1 gram per cu. ft.) gave a gas concentration of approximately 28 milligrams formaldehyde per cubic foot at 10 minutes after release of the gas.

(c) *The effect of formaldehyde on the developing chick embryo.*

Methods

Groups of newly-laid eggs, uncontaminated with *S. pullorum* were placed daily over a period of six days in standard forced draught incubator. The incubator was maintained at 100° F. dry bulb and 85° F. wet bulb during the course of the experiment. Three hours after the last group of 102 eggs had been added, the incubator was completely sealed and all groups of eggs fumigated with formaldehyde in a concentration of 22 mg. per cu. ft. at 20 minutes. After 20 minutes' fumigation, the incubator ventilators were opened and incubation was continued for a further three days. At the end of this period, all eggs were individually opened and the embryos carefully examined.

The eggs of one group (E) were fumigated prior to incubation for a period of 60 minutes, the formaldehyde gas concentration at the end of the first 20 minutes being 25 milligrams per cubic foot. After fumigation these eggs were placed in the above mentioned incubator and incubated for three days, after which they were subjected to a second fumigation along with the other groups of eggs.

XUM

Results

These are shown in Table I. It was difficult to assess the true significance of some of the mortality groups. It is clear that in all the groups of eggs which had been incubated prior to fumigation, there were a number of embryos which had died before fumigation. For instance, in the group incubated two days before fumigation, 10 embryos died before the eggs were fumigated. Thus it is impossible to determine, in the case of the group of eggs incubated three hours before fumigation, the number of embryos which died as a result of the fumigation itself.

TABLE I

The effect of formaldehyde on embryos of varying ages

No. of eggs	Age of embryo when fumigated	No. of embryos alive 3 days after fumigation	No. of eggs clear	No. of embryos dead	Age of embryos at death
66	6 days	49	9	8	4 died within 48 hours 2 at approx. 4th day 1 „ „ 5th day 1 „ „ 6th day
66	5 days	54 (one with one eye)	6	6	3 died 0-48 hours 1 „ at approx. 3rd day 1 „ „ „ 4th day 1 „ „ „ 6th day (this embryo had 2 heads)
66	4 days	42	13	11	5 died 0-48 hours 1 „ at approx. 3 days 2 „ „ „ 4 days 2 „ „ „ 5 days 1 „ „ „ 8 days
66	3 days	49	6	10	6 died 0-48 hours 1 „ at approx. 2 days 3 „ „ „ 3-4 days
66	3 days (Group E fumigated twice)	47	10	9	4 died 0-48 hours 3 „ at approx. 2 days 1 „ „ „ 3-4 days 1 „ „ „ 5-6 days
62	2 days	34	11	18	10 very early dead 7 died approx. 2 days 1 „ „ 4 days
64	24 hrs	54	8	4	3 very early dead 1 died at approx. 2 days
102	3 hrs	70	22	10	All died 0-36 hours

If the mortality figures are related to the age of the embryo at time of fumigation, it will be seen that the highest mortality occurred in the group which were two days old at the time of fumigation. It would appear that seven embryos in this group out of a total of 51 fertile eggs died at the time of fumigation. The group of eggs which had been subjected to two fumigations (Group E) showed no mortality which might have been attributed to the fumigation.

In a second experiment, the effect of a known concentration of formaldehyde was tested on embryos of six to 19 days' incubation. Eggs were placed in the incubator at intervals of a few days and six days after the last group of eggs was added, the incubator was sealed and all eggs were fumigated. The resulting gas concentration at 20 minutes was 22 milligrams formaldehyde per cubic foot. Following this 20 minute fumigation, the incubator ventilators were

TABLE II

The effect of formaldehyde on embryos of varying ages

No. of Live embryos 4 days before fumigation	Age of embryos at time of fumigation	No. hatched	No. dead	Age at death
30	19 days	22	8	2 died at 15 days 2 died at 11 days 4 died at full term
35	17 days	29	6	2 died at 15 days 4 died at full term
31	14 days	21	10	2 died at 12 days 2 died at 15 days 2 died at 19 days 4 died at full term
23	12 days	16	7	2 died at 8 days 2 died at 19 days 3 died at full term
39	9 days	28	11	2 died at 8-9 days 1 died at 7 days 2 died at 15 days 3 died at 19 days 3 died at full term
91	6 days	72	19	2 died at 9 days 2 died at 13 days 2 died at 15 days 1 died at 18 days 8 died at 19 days 4 died at full term

opened and normal incubator management was continued until all chicks had hatched.

The results which are shown in Table II and which relate to embryos six to 19 days old, indicate that there was no undue embryonic mortality which could be attributed to the fumigation of embryos of these ages.

It will be seen that Table II extends the results given in Table I which was confined to embryos up to six days' incubation.

Observations carried out in the Field

The opportunity has been taken to carry out a number of observations at two large hatcheries where pr incubation fumigation at room temperature is carried out.

In the first hatchery, the loaded egg trays were stacked in racks in a wooden cabinet, the front of which was closed by a plastic curtain. After drawing the curtain, formalin was added to potassium permanganate in shallow trays placed on the floor. There was no mechanical means of dispersing the formaldehyde.

The racks held approximately 13,000 eggs, and the total capacity was approximately 200 cubic feet. A total of 3 oz. formalin and 2 oz. potassium permanganate was divided between two shallow trays, i.e. 1½ oz. to 1 oz. per 100 cu. ft. (0.42 c.c. to 0.283 grams per cu. ft.).

Gas samples were taken in the usual manner and the results are given in Table III.

These results would indicate that the amount of formalin used was inadequate. The exceptionally low reading found at floor level suggests a disadvantage in this particular type of construction.

TABLE III

Position in the block of trays, of the end of the capillary lead	Time in minutes, after introduction of the formalin, during which gas samples were taken	Concentration of formaldehyde mg. per cu. ft.
Towards the back of the uppermost tray of eggs	5-10	8.0 mg.
	30-35	10.1 mg.
Tray midway from floor	5-10	19.4 mg.
	30-35	14.3 mg.
Tray near floor	5-10	Nil
	30-35	1.6 mg.

In the second hatchery an air-tight chamber had been constructed with a ventilation system which provided for rapid recirculation of air in the chamber and hence adequate mixing of the gas generated. The volume of the chamber was approximately 250 cubic feet.

Three fumigations were carried out, each using 3 oz. formalin to 2 oz. potassium permanganate per 100 cu. ft. (0.84 c.c. to 0.566 grams per cu. ft.). In each case capillary leads were placed in the following positions.

Position A.—In the centre of the three trolleys of eggs.

Position B.—In the centre of the free space immediately above the top egg tray.

Position C.—In the centre of the chamber on top of the second lowest egg tray.

The results of the various gas analyses made are given in Table IV.

TABLE IV

Fumigation	Position of end of capillary lead	Time in minutes, after release of formaldehyde, during which gas samples were taken	Concentration of formaldehyde (mg. per cu. ft.)
1	Position A	1-6	69.4
	Position B	1-6	57.9
	Position C	1-6	57.9
2	Position A	1-6	59.9
		15-20	8.8
	Position B	1-6	69.4
		15-20	8.0
	Position C	15-20	7.0
3	Position A	15-20	13.8
	Position B	15-20	11.1
	Position C	15-20	11.1

In carrying out the first of these observations, leakage of formaldehyde from the chamber was noted. The second and third series of observations were made after temporary alterations had been made to limit the amount of gas escaping. The gas concentrations, especially those taken at 15-20 minutes, must therefore be lower than those which would be obtained with a completely gas-tight chamber. It was considered that by rendering the chamber completely gas-tight and by increasing the amount of formalin to 4 oz. per 100 cubic feet (1.13 c.cs. per cu. ft.) an adequate concentration of formaldehyde would be produced.

Discussion

The authors are unaware of any publication previous to this series in which the formaldehyde gas concentration has been related to its bactericidal efficiency against *S. pullorum*. It was of considerable interest to find that in the absence of additional temperature and humidity, approximately three times the concentration of formaldehyde was required to kill *S. pullorum* on the surface of the hen's egg. It has been shown in the previous publications in this series that, at normal incubator temperature and humidity, a minimum level of 6.5 milligrams of formaldehyde per cubic foot was required at 20 minutes to kill *S. pullorum* completely. In a completely sealed incubator under these conditions 1½ oz. formalin per 100 cubic feet (0.42 c.c. per cu. ft.) resulted in a gas concentration of 10 milligrams formaldehyde per cubic foot. When fumi-

gation is carried out at room temperature and humidity, a minimum gas concentration of 17 milligrams per cubic foot at 20 minutes was required. This gas concentration resulted from the use of $3\frac{1}{2}$ oz. formalin per 100 cubic feet (1 c.c. per cu. ft.). For practical purposes it is considered that 4 oz. formalin per 100 cubic feet (1.2 c.c. per cu. ft.) should be adequate for the fumigation of eggs in an airtight chamber maintained at normal atmospheric temperature and humidity. These findings are in agreement with Wilson (1951) who found that, under ordinary atmospheric conditions, 1.5 c.c. formalin per cubic foot ($5\frac{1}{4}$ oz. per 100 cu. ft.) was invariably lethal to *S. typhi-murium* on egg shells.

Several workers have reported the effect of formaldehyde on embryonic mortality. Burton (1946) indicated that embryos should not be exposed to high strengths of formaldehyde during 24th-28th hours of incubation. Wilson (1951), however, found that embryos of 23 hours incubation appeared quite unaffected by formaldehyde. In the work now being reported, it was impossible to include unfumigated eggs for control purposes. To do this either a separate incubator would have had to be used or the same incubator used at a subsequent date. Neither of these alternatives would have formed adequate control procedures. In interpreting the results of Table I it must be noted that of the eggs which were 48 hours old at the time of fumigation, ten embryos died prior to fumigation. This figure is approximately double the pre-fumigation deaths which occurred in the other age groups.

The present results, however, appear to indicate that embryos of 48 hours incubation are particularly susceptible and the effects of formaldehyde fumigation may extend to embryos up to five days of incubation. On the other hand, as shown in Table II, fumigation caused no undue mortality in embryos of six days and older.

There appeared to be little or no adverse effect on developing embryos when fertile eggs were subjected to high concentrations (22 milligrams per cubic foot) of formaldehyde prior to incubation. It is readily appreciated that the effect of pre-incubation fumigation on hatchability will have to be repeated on far greater numbers of eggs than was possible during this work.

Summary

1. The effectiveness of formaldehyde fumigation when carried out in airtight chambers maintained at atmospheric temperatures and humidity has been studied.

2. When fumigation is carried out for a period of 20 minutes, $3\frac{1}{2}$ oz. formalin per 100 cubic feet capacity (1 c.c. per cu. ft.) produced a gas concentration (14 milligrams formaldehyde per cubic foot), sufficient to kill *S. pullorum*, on the surface of the hen's egg. In practice, 4 oz. per 100 cu. ft. (1.2 c.c. per cu. ft.) is advised for the purpose. To kill *S. pullorum* after 10 minutes exposure (minimum gas concentration 25 milligrams formaldehyde per cubic foot) $5\frac{1}{4}$ oz. formalin per 100 cubic feet (1.5 c.c. per cu. ft.) is required.

3. The practical application of formaldehyde estimation in the field is

described. The results show that leakage and poor distribution of the liberated gas were important factors influencing the efficiency of the pre-incubation fumigation equipment of the two commercial hatcheries concerned in this report.

4. The hatchability of fertile eggs did not appear to be affected when fumigated prior to incubation for 60 minutes at a concentration of 22 milligrams formaldehyde per cubic foot at 20 minutes.

Acknowledgments

We wish to thank Miss Sheila Day for her technical assistance; Mr. C. G. Stacey for the supply of experimental material and the owners of the two hatcheries for the facilities granted. This work was carried out with the assistance of a grant from the Agricultural Research Council.

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RATE OF HATCHABILITY OF TUBERCULOUS ARTIFICIALLY INFECTED EGGS

By M. NAGUIB, M.V.Sc. (Cairo)

Introduction

It has been established by many workers that eggs harbouring tubercle bacilli may produce tuberculous chicks.

Maffucci (1892) inoculated eggs with avian tubercle bacilli and found that tuberculosis developed in the majority of chicks. Fitch and Lubbehusen (1928) found that 14 chicks were hatched out of 78 eggs inoculated with avian tubercle bacilli; 13 chicks died within fifteen days and one survived for four months. All were found to be tuberculous. They also showed an increase in the number of dead germs (i.e. embryos) in the unhatched eggs. Wahby (1929) demonstrated that the hatchability of artificially infected eggs is poor but such eggs may hatch and chicks are short-lived and affected with tuberculosis. Thorp and Graham (1930) showed that artificially inoculated eggs had a lower hatchability and a higher mortality of the embryo than was the case in the control eggs. Klimmer (1930) after reviewing Wahby's data maintained that the presence of tubercle bacilli in fertilised eggs influenced adversely the development of the embryo and decreased the hatchability. He noted also the short duration of life of infected chicks.

XUM

Experimental Data

Eggs were collected from 35 healthy hens with which 5 cocks were housed. A suspension of virulent avian tubercle bacilli was prepared in normal saline solution and the concentration of avian tubercle bacilli in this stock suspension was determined.

Four dilutions, namely 1 :10, 1 :100, 1 :1,000 and 1 :2,000 were made of the stock suspension for the inoculation of eggs A hole was made into the dry, clean and disinfected egg shell at about 1.5 cm. from the pointed end of the egg to avoid puncturing the air sac or injuring the germinal disc; 0.01 c.c. of the required dilution was inoculated through this hole using a 20-gauge syringe needle. The hole was then sealed with collodion and the egg incubated at 39° C.

Experiment I

The stock suspension contained 106,633 avian tubercle bacilli per 0.01 c.c.

One-hundred-and-nineteen fertile eggs collected from the healthy hens were divided into six lots; each of 20 eggs except the second lot which was composed of 19 only.

Each egg of the first lot received 10,663, the second 1,066, the third 106 and the fourth 53 tubercle bacilli. The fifth lot was injected with 0.01 c.c. of sterile normal saline. The sixth was used as control and not injected. Each lot was incubated in a separate partition in order to keep both eggs and chicks under strict control.

Experiment II

Another experiment was repeated on the same lines on 120 eggs collected from the same hens. This stock suspension contained 102,177 tubercle bacilli per 0.01 c.c.

The first lot of eggs received 10,217, the second 1,021, the third 102 and the fourth 51 tubercle bacilli. The fifth and sixth lots were dealt with as in Experiment 1.

Results

The results of hatchability of both experiments are shown in Table I.

The eggs containing dead germs were bacteriologically examined for the presence of tubercle bacilli using Wahby's technique. The same technique of examination was followed in regard to both the fluid surrounding the dead chick and its internal organs in those eggs containing dead chicks.

All smears prepared of the sediment of the dissolved material obtained from all four infected groups revealed microscopically the presence of tubercle bacilli.

The hatched living chicks were leg-banded and marked with the number of the lot to which they belonged and they were fed and raised together. Their droppings were collected seven days after hatching and were subjected to bacteriological examination. No tubercle bacilli could be detected microscopically but luxuriant growth was obtained on Wahby's medium after three weeks.

The infected chicks died at varying intervals ranging from 2 to 23 days

TABLE I

Lot No.	No. of eggs Exper. I	Exper. II	Dead germs (embryos) Exper. I	Exper. II	% of dead germs Exper. I	Exper. II	Average percentage of dead germs	No. of chicks dead in shells Exper. I	Exper. II	No. of living chicks Exper. I	Exper. II	% of hatchability Exper. I	Exper. II	Average percentage of hatchability
1	20	20	17	16	85	80	82.5	3	3	0	1	0	5	2.5
2	19	20	15	15	78.9	75	76.9	3	3	1	2	5.2	10	7.6
3	20	20	14	13	70	65	67.5	4	3	2	4	10	20	15.0
4	20	20	13	11	65	55	60.0	4	4	3	5	15	25	20.0
5	20	20	8	9	40	45	42.5	5	3	7	8	35	40	37.5
6	20	20	4	6	20	30	25.0	4	2	12	12	60	60	60.0

TABLE II

Lot No.	No. of chicks	Age on death	Autopsy Findings	Micro-scope exam.	Cult-ural exam.
Experiment I.					
2	1	5	No macroscopic lesions	+	+
3	2	2	„ „ „	+	+
		10	„ „ „	+	+
		9	„ „ „	+	+
4	3	13	Congested liver with small nodules	+	+
		19	Liver and spleen congested enlarged with nodules	+	+
Experiment II.					
1	1	3	No macroscopic lesions	+	+
2	2	5	Congested liver and enlarged spleen	+	+
		10	No macroscopic lesions	+	+
		2	„ „ „	+	+
3	4	3	„ „ „	+	+
		15	Small focus in one lobe of liver	+	+
		18	Lesions in liver and spleen, size of pin head	+	+
		4	No macroscopic lesions	+	+
		5	„ „ „	+	+
4	5	14	„ „ „	+	+
		19	Small tubercles in liver and spleen	+	+
		23	Miliary tubercles in liver, spleen, intestines and lymph glands	+	+

+ = Microscopical or cultural examination revealed tubercle bacilli.

after hatching. They were autopsied immediately after death. Microscopical and cultural examinations of the internal organs especially livers, spleens and intestines were undertaken.

The results of post-mortem and bacteriological examinations of these chicks are tabulated in Table II.

To determine the possibility of transmission of tuberculosis among newly-hatched chicks inside the incubator the six chicks hatched from the artificially infected eggs in the first experiment were allowed to mix for two days in the incubator with the twelve healthy chicks which came from the control eggs and when they were due to come out of the incubator both lots were separated. No tuberculous infection among the healthy chicks resulted from this mixing inside the incubator.

Furthermore, to ascertain the sanitary importance of tuberculosis-transmission among newly-hatched chicks in the pen, the twelve chicks which had been hatched in the second experiment from normal non-infected eggs were put in the same pen together with the twelve infected chicks hatched from the infected eggs and when the later chicks died, the non-infected chicks were left in the same pen for three months. Tuberculosis developed in one of the healthy chicks. This represented 8.3 per cent of the healthy chicks.

Discussion of the Results

The experiments yielded information which throws some light on the sanitary aspect of avian tuberculosis.

It was proved that eggs, artificially infected with avian tubercle bacilli, could hatch and tuberculous chicks could be produced. As it is seen from Table I, the average hatchability percentage of lots 1, 2, 3 and 4 in both trials have been 2.5, 7.6, 15 and 20 respectively. Compared with the percentage of 60, for normal unhandled eggs, the hatchability percentages of infected eggs were very low. The decrease in hatchability was attributed to the presence of tubercle bacilli in the eggs before their incubation. It is interesting to observe that hatchability was inversely proportional to the number of tubercle bacilli injected into the eggs. In the first trial, six tuberculous chicks were obtained from 79 artificially infected eggs. These low figures of hatchability coincide with the results obtained by Fitch and Lubbehusen, Wahby, Thorp and Graham and Klimmer.

It was found also that manipulation and mechanical injury of eggs incidental to inoculation had an effect upon their hatchability. The average percentage of hatchability in normal unhandled eggs was 60, compared with 37.5 of eggs inoculated with normal saline.

The death rate of chicks in both trials was increased by both mechanical injury and tubercle bacilli but not to the same degree (see Table I). Identical results were obtained by Fitch and Lubbehusen, Wahby, Thorp and Graham and Klimmer. It was evident that the death rate became higher as the number of micro-organisms per inoculum was increased.

Newly-hatched chicks originating from healthy eggs did not contract

infection inside the incubator when mixed with infected chicks hatched from infected eggs for two days before being let out to the pen. On the other hand, infection was transmitted from the infected chicks to the healthy ones when they were mixed together in the same pen. In high probability the fæces were the factor of such transmission because they were proved by culture to contain living tubercle bacilli.

Summary

One-hundred-and-fifty-nine fertilised eggs were inoculated with varying numbers of avian tubercle bacilli, 40 eggs were inoculated with normal saline and 40 eggs left as control.

The average hatchability percentages of infected eggs were 2.5, 7.6, 15 and 20 and the dead germs were 82.5, 76.9, 67.5 and 60 according to the number of bacilli injected. The average percentage hatchability of eggs inoculated with normal saline was 37.5, while that of the control was 60. The percentage of dead germs (i.e. embryos) in the manipulated eggs was 42.5 compared with 25 in the control. The hatchability of eggs was lowered and the dead germs were increased by mechanical injury incidental to incubation. The more bacilli inoculated the less degree of hatchability.

Eighteen tuberculous chicks were obtained from 150 infected eggs and survived for varying periods; from 2 to 23 days. Infection of healthy chicks through the tuberculous newly-hatched chicks inside the incubator did not take place. Infection was transmitted to healthy chicks from the infected ones when mixed together in the same pen at a rate of 8.3 per cent.

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ABSTRACTS FROM CURRENT LITERATURE

ADVANCES IN ANIMAL NUTRITION. Mark Welsh and L. B. Tennison. *Veterinary Medicine, 49,* 191. May, 1954.

THE authors review the "phenomenal" advances made in recent years due to the introduction of antibiotics as a component of animal rations. Apart from improved growth and economic returns from livestock, use of the major antibiotics, Aureomycin, Penicillin, Streptomycin, Terramycin and Bacitracin, has, they say, brought the physiologist, biochemist, pathologist, veterinary surgeon and nutritionist into a common sphere of co-operation and mutual interest.

Brief results of further large scale field trials are given in tabular form and indications of the action of antibiotics in conditions of health and disease are

outlined together with suggested levels in feed for poultry and swine. A note is given of research to date on the use of antibiotics for ruminants. The authors claim that their recent work has shown that antibiotics may be of significant value in the fattening of beef steers and lambs.

D.C.

AN INVESTIGATION OF SUSPECTED RADIATION DAMAGE TO LIVE STOCK, by Brigadier-General J. L. Hartman, V.C., U.S. Army, and Lt.-Col. Bernard F. Trum, V.C., U.S. Army. University of Pennsylvania, Philadelphia, *Veterinary Extension Quarterly, 134.* April 9, 1954. 87.

IN Nevada, where in 1953 a series of atom bomb tests was made, several cows of a herd died of unknown causes which were supposed to be due to the fall-out of contaminated dust particles from the atmosphere. It was found that the radioactivity in the skin and in the carcases of some of the animals which had died, was not sufficient to account for death. It was, therefore, supposed that drought and subsequent lack of forage were the greatest contributing factors involved in the loss. The animals did not show skin lesions and cattle which in previous tests had received 10,000 roentgen physical equivalents over a large area of the body, had lived normally for several years.

Horses running wild on the range had evidently entered a heavy fall-out area during the recent tests, for they had numerous beta burns. They are still alive and appear to be normal except for the skin lesions. The blood tests* also were not indicative of radiation damage, although radiation hazard has to be carefully considered in such areas.

In suspected cases, therefore, a correlation of measured radioactivity and known biological responses should be demonstrable.

*In the ruminants.

H.R.

REVIEW

"PRACTICAL CLINICAL BIOCHEMISTRY," by H. Varley, 1954. Pp. 551. London: Heinemann (Medical Books) Ltd. Price 42s.

To a large extent American texts have tended to dominate the field of clinical biochemistry, so that the appearance of a new British book is an event of some interest to biochemists, pathologists and clinicians.

The author surveys the whole field of the subject "from the standpoint of workers in hospital laboratories" and hopes that it "will appeal particularly to registrars training in clinical pathology, to hospital biochemists, and to laboratory technicians." It may be said at the outset that he has very largely succeeded in his aims and that the new book compares favourably with well established and larger publications in the field, for its 28 chapters cover most of the examinations one is likely to make of blood, urine, and fæces, or of bodily function in its biochemical aspects.

The volume deserves to continue through further editions, for which reason the reviewer now wishes to indicate those portions of it which may, perhaps, be

improved, firstly from a general point of view and secondly from the standpoint of the veterinary biochemist.

Firstly, the introductory chapter would be better for further references to the errors associated with pipettes and volumetric apparatus: this applies more particularly to the use of the book by technicians and clinicians. Secondly, the number of methods given for the determination of blood sugars is no less than seven and even then does not include the use of anthrone. The existence of so many methods does not indicate a very satisfactory state of affairs Such a plethora of methods is certainly no fault of the author but, though he compares them briefly, in the end he says that "the actual choice of method may be influenced by the needs and circumstances of a particular laboratory," a statement which may not help a technician very much. Although the use of the elegant Conway micro-diffusion unit is described (p. 495), the apparatus is not indexed either under "Conway" or under "micro-diffusion."

From the veterinary point of view the book will be improved by (*a*) the enlargement of the chapter on "Milk," which is so sketchy (4pp.) as to be of little value; (*b*) the inclusion of methods for volatile fatty acids; and (*c*) by the addition to "Drugs and Poisons" of methods for the determination of arsenic and one or two other substances, e.g. ricin, which in ordinary circumstances, may affect livestock adversely. The chapters on "Vitamins" and "Hormones" seem to be adequate in a volume of this size.

Printing and binding of the book are good; and in conclusion it may be repeated that the book is a worthy effort.

NOTICES

GLAXO LABORATORIES, LTD. announce the introduction from May 17 of Streptopen Uterine Tablets, each tablet containing 500,000 units procaine penicillin and 0.5 gram streptomycin sulphate. These tablets are intended for local application in diseases of the uterine tract and in cases of retained fœtal membranes in animals. They are recommended after parturition in farm animals, particularly in assisted cases.

Streptopen Uterine Tablets are packed in tubes, each containing six tablets. The recommended usage is 1 tablet for cows and mares, ½ tablet for ewes, goats and sows.

MR. K. L. VAUGHAN, D.V.Sc., M.R.C.V.S., has been appointed manager of the Veterinary Information Department of Evans Medical Supplies, Ltd., Speke, Liverpool.

THE LATE PROFESSOR J. B. BUXTON

It was with great regret that we learnt of the passing of Professor J. B. Buxton, Principal and Dean of the Royal Veterinary College, London. He died at his desk on May 25. We take this opportunity of tendering our deepest sympathy immediately to Mrs. Buxton and the family. It is our intention to pay a tribute to his life and work in a subsequent issue.